In the Title (Clean Copy)

METHOD FOR SCREENING SUBSTANCES CAPABLE OF MODULATING THE ACTIVITY OF A TRAAK POTASSIUM CHANNEL

In the Specification (Clean Copy)

On page 4, please replace the fourth through the sixth paragraphs with the following:

Fig. 4 shows the electrophysiological properties of the TRAAK currents recorded using the imposed voltage technique on Xenopus oocytes that had received an injection of TRAAK cRNA (a, b, c) and on COS cells transfected with a vector expressing TRAAK (d, e).

Figs. 5a and b are graphs showing the effect of the osmolarity of the external medium on oocytes that received an injection of TREK-1 or TASK cRNA.

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Figs. 6a - h are graphs showing that TREK-1 is a mechanosensitive potassium channel in the transfected COS cells.

On page 4, please replace the eighth through the tenth paragraphs with the following:

Figs. 8a - f are graphs showing the activation of TREK-1 by arachidonic acid in the transfected COS cells.

Figs. 9a - e are graphs showing the effect of arachidonic acid and other fatty acids on the TRAAK channel expressed in the transfected COS cells.

Figs. 10a and b are graphs showing the effect of riluzole on the TREK-1 and TRAAK designated TREK-2 currents.

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On page 6, please replace the second paragraph with the following:

Such derivatives include those with a sequence comprising a modification and/or a suppression and/or an addition of one or more amino acid residues, as long as this modification and/or suppression and/or addition does not modify the properties of the TRAAK channel. Such derivatives can be analyzed by the expert in the field using the techniques described in the examples presented below which enable demonstration of the biophysical and pharmacological properties of the TRAAK channel. More specifically, such a derivative is the TREK-1 channel the amino acid sequence of which is represented in the attached sequence list as SEQ ID No.: 4.

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On page 7, please replace the first full paragraph with the following:

Another nucleic acid sequence according to the invention comprising at least one sequence coding for the protein constituting the TREK-1 channel which has the amino acid sequence represented in the attached sequence list as SEQ ID No: 2 or for a functionally equivalent derivative of this protein. A DNA molecule comprising the sequence coding for the TREK-1 protein is represented in the attached sequence list as SEQ ID No: 2 or its complementary sequence. More specifically, such an amino acid sequence comprises the sequence between nucleotides 484 and 1596 of SEQ ID No.: 2.

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On page 11, please replace the first full paragraph with the following:

Fig. 5 shows the effect of the osmolarity of the external medium on oocytes that received an injection of TREK-1 or TASK cRNA. In Fig. 5a: comparison of the effects of the application of a hypertonic solution (417 mOsm, by addition of mannitol) on control oocytes (CD8) and on oocytes expressing TASK or TREK-1 are shown. The currents were measured after a potential jump from -80 to +80 mV. The inset shows the TREK-1 current before and after (indicated by an arrow) the application of the hypertonic solution. In Fig. 5b: reversible effect of a hypertonic solution (434 mOsm, by addition of sucrose) on the current-potential relations deduced from the potential ramps which lasted 600 msec is shown. The inset shows the kinetics of the effect produced by the hypertonic solution. The currents were measured at 80 mV.

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Please replace the paragraph spanning pages 11 and 12 with the following:

Fig. 6 shows that TREK-1 is a mechanosensitive potassium channel in the transfected COS cells. In Fig. 6a: channel activities (N*Po) in the membrane patches were maintained at 0 mV and obtained in the attached cell configuration from control cells (CD8) or from cells transfected by TREK-1 and TASK. In Fig. 6b: stretching the membrane had no effect on the activity of the TASK channel (attached cell configuration). The patch was maintained at 50 mV. In Fig. 6c: the TREK-1 channels were silent at rest and opened upon tension of the membrane. The patch was maintained

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at +50 mV. In Fig. 6d: the histogram shows the amplitude of the channel activity generated by the membrane tension and illustrated in Fig. 6f. In Fig. 6e: current-potential relation in a single TREK-1 channel (n = 6) is seen. The conductance of 81 pS, was calculated between 0 and 80 mV. In Fig. 6f: activation of TREK-1 by stretching the membrane (30 mmHg) in the inside-out configuration

B6 is shown. The maintenance potential was 100 mV. In Fig. 6g: effects produced by higher and higher tensions (5 seconds duration) on the current-potential relation of a patch expressing TREK-1 are shown. In Fig. 6h: dose-effect curve of the activation of TREK-1 by the tension (n = 6) is seen. The curve was traced by following the experimental points according to the Boltzmann relation.

Please replace the paragraph spanning pages 12 and 13 with the following:

Fig. 8 shows the activation of TREK-1 by arachidonic acid in the transfected COS cells. In Fig. 8a: the activity of TREK-1 was recorded in the attached cell configuration. The patch was stimulated by a potential ramp lasting 800 msec every 5 seconds. The currents were measured at 80 mV. The applications of arachidonic acid (AA, 10 μM) are indicated by the horizontal bars. During the experiment, the patch was stimulated by tensions of 50 mmHg (indicated by the arrows). At 9 minutes, the patch was excised in the inside-out configuration. In Fig. 8b: current-potential relations corresponding to the experiment illustrated in Fig. 8a is shown. In Fig. 8c: activity of TREK-1 in

B7 the attached cell configuration with 10 μM AA in the pipette can be seen. The potential ramp lasted 800 msec and the currents were measured at 80 mV. In Fig. 8d: single-channel current-potential relations at the moment at which the pipette was placed on the membrane or after 20 minutes and 1 minute after the patch was excised in the inside-out configuration. In Fig. 8e: effect of AA (10 μM) on the TREK-1 current recorded in the intact cell is demonstrated. The current was measured at 80 mV. In Fig. 8f: AA had no effect on the TREK-1 current measured in the intact cell when it was in the pipette. The current was measured 30 minutes after the patch was broken (control tracing) by a potential ramp of 800 msec. The current was then measured after an application of AA of 1 minute in the external medium (AA tracing).

On page 13, please replace the first and second full paragraphs with the following:

Fig. 9 shows the effect of arachidonic acid and other fatty acids on the TRAAK channel expressed in the transfected COS cells. In Fig. 9a: current-potential relations obtained from potential ramps of 5W msec from -150 to +50 mV, after application of AA (10 μM) and after washing are shown. The inset shows the currents triggered by the potential jumps from -130 to +50 mV in increments of 20 mV. The maintenance potential was -80 mV. In Fig. 9b: dose-effect relation of the activation of TRAAK by AA is shown. In Fig. 9c: current-potential relations obtained as in Fig. 9a in the outside-out configuration are shown. The inset shows the effect of AA at 20 mV. In Fig. 9d: a histogram represents the coefficient of augmentation of the currents obtained after application of various fatty acids (10 μM). In Fig. 9e: the histogram shows the value of the currents recorded in the intact cell configuration before and after application of AA on the cells temporarily transfected by TWIK-1, TASK, TREK-1 and TRAAK and on the cells transfected in a stable manner by TRAAK. The coefficient of augmentation is indicated in each case.

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Figs. 10a and b are graphs showing the effect of riluzole on the TREK-1 and TRAAK designated TREK-2 currents. The current-potential relations were obtained as in Fig. 9a above and after application of riluzole (100 μM) on the transfected COS cells. The inset shows the effects of riluzole on the currents recorded in the outside-out configuration.